

The University of Chicago

VERNALIZATION
OF MARQUIS WHEAT AND OTHER
SPRING CEREALS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1940

By
DENNIS JAMES WORT

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VERNALIZATION OF MARQUIS WHEAT AND OTHER SPRING CEREALS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 507

D. J. WORT

(WITH SIX FIGURES)

Introduction

In 1933, WHYTE and HUDSON (22) presented a translation and compilation of the literature on vernalization of plants. A bulletin (9) appeared in 1935 which summarized the research on this subject in the Soviet Union and twenty-six other countries. Many aspects of vernalization, theoretical and practical, have recently been discussed by WHYTE (21).

There is great diversity of opinion with respect to the reaction of spring cereals to vernalization. Data collected by FOKEEV and VYROV (3, 4) from various places in the U.S.S.R., where extensive use of vernalization has been made, showed 2 to 4 days earlier emergence of vernalized seedlings from the soil. Vernalization stimulated root development, which in turn led to increased development of green parts. The thicker stand, which was maintained throughout the vegetative season, reduced weed growth. At harvest vernalized plants were on the average 9.5 cm. taller. Vernalized grain began earing 2.5 days earlier than controls. In dry districts there was a recorded increase in grain yield of 28.6 per cent as compared with 17-18 per cent in other districts. The quality of the wheat grain was also improved. ZENJUK (24) grew vernalized wheat at the Minsk Marshland Station to determine the acceleration of maturity. In later sowings (May 3) vernalization accelerated the onset of all developmental phases, whereas in early sowings the difference was evident only prior to ear emergence.

VAN HOEK (20), working in Holland, vernalized spring wheat for 7 days and for 16 days, at 3°-6° C. The 16-day treatment was the more successful, resulting in an acceleration of earing of 3 days and of maturity of 1-4 days. GMELIN (6), also working in Holland and

using the same variety of wheat as VAN HOEK, could obtain no acceleration of maturity. In Rumania, PIESCU (16) found that vernalized spring varieties headed at the same time as controls.

In the United States, MCKINNEY *et al.* (15) grew vernalized Marquis wheat at Langdon, North Dakota, 20 miles south of the Canadian boundary. No decreases in time to mature were obtained, but treatment for 10 days gave average increases in yield of 19.07 per cent over unchilled, ungerminated controls. TAYLOR and COFFMAN (19) vernalized Isogold, a variety of spring oats. Treatment retarded heading by 2 days and gave a decrease of 37 per cent in yield. This decrease is ascribed to less germination, since the stand in the vernalized plot for a period of 4 years averaged only 51 per cent of that in the untreated plots.

Referring to experimental data, KONSTANTINOV (10) asserts that although vernalization of cereals, maize, millet, sunflower, Sudan grass, and other crops is widely used in all parts of the Soviet Union, the efficacy of vernalization is closely connected with the region and after-sowing conditions. The cold spring of southeast Russia is rather unfavorable for vernalized sowings, or at least less favorable than the warm, moist, prolonged spring of certain other regions. The efficacy of vernalization varies also with the time of sowing, later sowings being more beneficial.

The chief edaphic difference between the early and late plantings, particularly at high latitudes, would be soil temperature, which would show an increase as the spring advanced. The preceding results therefore point to a possible correlation between soil temperature and efficacy of vernalization.

The phasic development theory assumes that plants can enter upon the next stages of development, given appropriate conditions, only when the previous stage has been completed. It is therefore of special importance to be able to determine whether a certain stage has been completed. Assuming that vernalization produces a change in the physico-chemical state of the protoplasm, BASSARSKAJA (1, 2) was able to detect the completion of the thermo-stage by the precipitation of Prussian blue within the tissues of the vernalized embryo upon addition of ferric chloride and potassium ferrocyanide. RICHTER (18) and GAVRILOVA (5) have used a staining technique

based upon the displacement of the iso-electric point of the tissues of the embryo to determine completion of the thermo-stage in cotton and wheat. A modification of the microchemical technique used by these investigators was employed in the diagnosis of vernalization of Marquis wheat.

In the following experiments, which were begun in 1934, the reaction of vernalized spring wheat, oats, barley, and rye with respect to vegetative period, yield, photoperiodic response, and growth at various soil temperatures was investigated. A preliminary attempt to use microchemical methods to diagnose the vernalized state is also described.

General method and terminology

Methods of vernalization are based primarily on the researches of LYSENKO (13). The general procedure for vernalization of all cereals consists in germinating the seeds for 24 hours at 15° – 20° C. and then transferring the germinated grain to low temperatures for a period, allowing the young plant to continue development without increasing in size.

For convenience a formula is employed to express the combinations of factors employed in the vernalization treatment. The factors and their units are: (1) length of time of chilling in days; (2) temperature during chilling in degrees Centigrade; (3) amount of water added in percentage of original air-dry weight of the seed. For example, the formula 8:3:60 means that the seed was chilled for 8 days at 3° C. following germination, the water added being 60 per cent of the original air-dry weight of the seed.

The vernalization method used consists of four parts:

1. The amount of water was added to the seeds in petri dishes.
2. The imbibed seeds were allowed to germinate until radicles had pierced the seed coats of a few grains (usually within 24 hours at 15° – 20° C.).
3. The germinated seeds were placed in the chilling chamber for the number of days indicated.
4. The covers of the petri dishes were removed and the seeds aerated for a few seconds each day.

Any departures from this method, necessary in the treatment of large quantities of seed, are noted in the text.

Investigation

EFFECT OF VERNALIZATION ON LENGTH
OF VEGETATIVE PERIOD AND YIELD

A. FIELD EXPERIMENTS

Investigations covering the period 1934 to 1937 were conducted at Saskatoon, Saskatchewan (52° N lat., 106° W long.), while the work of 1939 was performed at Chicago, Illinois.

TABLE 1

TREATMENT, HARVEST DATA, ETC., FOR FIELD EXPERIMENTS, 1934-1939

YEAR	GRAIN	VERNALIZATION FORMULA	WEIGHT OF SEED TREAT- ED	AREA SOWN	TREATMENT OF CONTROLS	AC- CELER- ATION OF FLOW- ERING (DAYS)	AC- CELER- ATION OF RIPEN- ING (DAYS)	IN- CREASED YIELD DUE TO VERNAL- IZATION	DATE OF PLANT- ING
1934	Marquis wheat	5	30 gm.	Five rows	Unchilled, un- germinated " " " " "	See table 2			May 5
	O.A.C. barley	7	"	4' long					"
	Hannchen bar- ley	13	"	"					"
	Banner oats	16:3:33	"	"					"
	Victory oats	17	"	"					"
		18	"	"					
		23	"	"					
1935	Marquis wheat	12:3:33	300 lb.	5 acres	"	0	0	21.04%	May 6
1936	Marquis wheat	12:3:33	60 lb.	1 acre	"	0	0	14.04%	May 7
1937	Marquis wheat	12:3:33	60 lb.	1 acre	(a) Unchilled, un- germinated				May 9
	Banner oats	12:3:33	20 lb.	$\frac{1}{2}$ acre	(b) Germinated As for wheat, 1937				May 9
1939	Marquis wheat	8:3:60	20 gm.	Seven rows 5'3" long	Germinated	2.3			July 3

Chilling: 1935, in room cooled by snow; others by mechanical refrigeration.

Planting: 1934, 1939, by hand; others by seed drill.

Location: 1939, in Chicago; others in Saskatoon, Sask.

The formula used during the years 1934 to 1937 was that of LYSENKO, with respect to amount of water added and temperature during treatment. Various lengths of treatment (table 1) were used in 1934 in an attempt to determine the optimum combination of the several vernalization factors used. The formula used in 1939, 8:3:60, was the one developed in 1938 which gave the greatest acceleration of flowering of Marquis wheat grown in the greenhouse during that year.

A modification of the method just given was necessary to vernalize large quantities of seed. The water was added in three equal amounts, 4 hours apart, and the seeds were stirred periodically to insure uniform moistening.

In 1934 the chilling chamber was a basement room, snow serving effectively to maintain an average temperature of 3° C. During chilling, the grain, which had been spread in a layer 6 inches deep on the floor, was mixed thoroughly once a day to insure adequate aeration. The 60-pound and 20-pound lots treated in 1935, 1936, and 1937 were chilled in bags which allowed a relatively free access of air. In all cases the treated grain was sown, without being dried, on the day it was removed from the refrigeration room. When more than one formula was used all treatments were concluded at the same time.

When the seed drill was used, setting of the drill at 2 bushels per acre and 1 bushel per acre sowed equal dry weights of vernalized and ungerminated, unchilled control grain, respectively. The setting had to be increased slightly beyond 2 bushels in the case of the germinated control.

In the 1935 and 1936 experiments the plots were sampled by harvesting 50 areas of 1 square yard each, taken in regular order throughout the plots. The field plots of treated and control grain were one-quarter mile long and located side by side. The soil conditions in the plots were practically identical.

The experiments of 1934 to 1937, in which 33 per cent water was added to the original dry weight of the seed, gave little acceleration of maturity of Marquis wheat, Banner and Victory oats, and O.A.C. barley, regardless of duration of treatment. In the case of Hannchen barley, however, 5:3:33 and 13:3:33 both resulted in a 7-day advance in flowering (table 2). The formula 8:3:60, used in 1939, accelerated flowering of Marquis wheat in the field by an average of 2.3 days.

Increases in yield were general, amounting to 100 per cent over control in the case of Banner oats, vernalized according to the formula 23:3:33, in the small-scale trial of 1934. The large-scale sowings of 1935 and 1936 gave yield increases by vernalized Marquis wheat of 21.04 and 14.04 per cent, respectively, over the untreated controls. In 1935 the vernalized Marquis wheat showed an increase

in height of 7.2 per cent, an increase in number of fertile spikelets per head of 5.0 per cent, an increase in average kernels per head of 2.5 per cent, and an increased 1000-grain weight of 18.64 per cent.

Drought during the spring and summer of 1936 resulted in a smaller increase in yield due to vernalization than in 1935, when growing conditions were more favorable. Vernalized Marquis wheat did not appear to have any greater or less resistance to the extreme drought of 1937, which caused complete crop failure. In 1939 a week

TABLE 2
ACCELERATION IN DAYS TO FLOWER, TO RIPEN, AND INCREASED YIELD DUE
TO VERNALIZATION. FIELD EXPERIMENT, 1934

DAYS VERNAL- IZED	ACCELERATION IN DAYS TO FLOWER OVER CONTROL					ACCELERATION IN DAYS TO RIPEN OVER CONTROL					PERCENTAGE INCREASED YIELD OVER CONTROL				
	MARQUIS WHEAT	O.A.C. BAR- LEY	HANNCHEN BARLEY	BANNER OATS	VICTORY OATS	MARQUIS WHEAT	O.A.C. BAR- LEY	HANNCHEN BARLEY	BANNER OATS	VICTORY OATS	MARQUIS WHEAT	O.A.C. BAR- LEY	HANNCHEN BARLEY	BANNER OATS	VICTORY OATS
5.....	2					0					20.8	64.3	74.3	70.4	50.0
7.....	-3*	-1	0	-4	0	-1	-1	5	-2	-1	14.6	52.3	37.3	50.0	50.3
13.....	0	1	1	1	0	0	1	5	0	1	5.2	54.0	46.1	95.0	31.2
16.....	-3	1	1	1	-1	-1	1	4	0	-1	-3.1	3.2	20.4	70.3	22.1
17.....	-3	0	0	0	-1	-1	1	2	-1	0	7.3	31.0	22.2	48.1	19.8
18.....	3	-1	0	1	-2	0	-1	2	0	-2	9.4	51.2	29.6	62.0	25.0
23.....	2	0	3	-1	0	0	1	2	-1	-1	27.1	74.1	37.3	100.0	25.0

* Minus sign indicates retardation in flowering or ripening.

of hot dry weather immediately following planting reduced germination of vernalized seeds of Marquis wheat 28.5 per cent more than the reduction in germination suffered by the control grain. At the end of this week the plots were watered and watering was continued periodically throughout the experiment. In spite of the greater reduction in germination, the vernalized plants responded to irrigation by producing 47.5 per cent more heads than controls in 87 days, the period of the experiment.

B. GREENHOUSE EXPERIMENTS

Experiment 1.—The results of the experiments concerning the photoperiodic response of various vernalized cereals, described in a later section, indicated the necessity for developing a formula for treatment, specific for each cereal. An attempt was made at Chicago

during the summer of 1938 to determine the vernalization formula for Marquis wheat which would give the shortest vegetative period. The experiment was begun June 21 and terminated August 26.

The possible combinations, 125 in all, of the following vernalization factors were employed:

1. Length of vernalization period: 5, 8, 11, 14, 17 days.
2. Temperature during vernalization: 3°, 5°, 7°, 10°, 13° C.
3. Water added: 20, 30, 40, 50, 60 per cent.

Two complete series of these various combinations were run as indicated in the following paragraphs. Forty-gm. lots of Marquis wheat were treated, using the procedure outlined at the beginning of this report and the 125 combinations of factors just given.

Series A consisted of lots of seed started at the same time and removed from the mechanical refrigerators at the end of 5, 8, 11, and 14 days. The removals and plantings were on June 27, June 30, July 3, July 6, and July 9. Series B consisted of lots of seed placed in the refrigeration chambers at such times that all the periods of treatment, including 17 days, were completed on July 9. Thus only series B included seed vernalized for 17 days.

Three plants were grown in 6-inch pots filled with loam soil. The control was grain to which water equal to 40 per cent of the dry weight had been added and allowed to germinate 24 hours at 20° C. No control over the temperature of the greenhouse was attempted. This rose nearly every day to 100° F. and fell at night to 70° F.

1. Effect of drying seed subsequent to treatment.—The percentage germination of the grain prior to vernalization was 94.75 and immediately after treatment 95.0. After being air-dried for 3 days following vernalization the germination was 86.0 per cent. Drying the vernalized grain decreased germination by approximately 9.5 per cent.

2. Length of vegetative period.—In table 3 only those combinations of length of vernalization, temperature, and percentage water added which resulted in a decrease in length of the vegetative period, compared with controls, in both series A and B are given. No results complying with this condition were obtained with 14-day treatment although decreases in time to flower were obtained in several cases in series A.

The combination of vernalizing factors which gave the earliest flowering of Marquis wheat under greenhouse conditions was 8:3:60. This formula gave an acceleration of 24 days in series A and 18 days in series B, or an average of 21 days in comparison with controls. In both cases the grain was ripe (the head showing no green color) or would have ripened in 50 days after planting. The formulas 8:10:50 and 8:13:20 produced an acceleration in flowering of 14.5 and 13

TABLE 3

COMBINATIONS OF VERNALIZING FACTORS GIVING DECREASES IN VEGETATIVE PERIODS IN SERIES A AND B, 1938, GROWN IN GREENHOUSE

VERNAL- IZATION FORMULA	SERIES A						SERIES B					
	DAYS FOR TREAT- ED PLANTS TO			CONTROL*		ACCELER- ATION IN DAYS TO FLOWER DUE TO VERNALI- ZATION	DAYS FOR TREAT- ED PLANTS TO			CONTROL*		ACCELER- ATION IN DAYS TO FLOWER DUE TO VERNALI- ZATION
	HEAD	FLOW- ER	RIP- EN	HEAD	FLOW- ER		HEAD	FLOW- ER	RIP- EN	HEAD	FLOW- ER	
5:3:50....	39	42	60	47	47	5	41	41		48	49	8
5:5:50....	43	46		47	47	1	40	40		48	49	9
5:13:60....	43	45	60	47	47	2	30	32		48	49	17
8:3:50....	43	44		55	56	12	40	41		48	49	8
8:3:60....	31	32	50	55	56	24	30	31	†	48	49	18
8:10:40....	47	48		55	56	8	33	33		48	49	16
8:10:50....	44	44		55	56	12	30	32		48	49	17
8:13:20....	45	46		55	56	10	30	33		48	49	16
8:13:60....	48	48		55	56	8	41	41		48	49	8
11:3:30....	48	48		52	53	5	33	34		48	49	15

* No controls ripe August 26, when experiment was terminated.

† Nearly ripe in 48 days.

days, respectively; 17:5:60 and 17:7:40 gave an acceleration of 17 days in flowering compared with their controls, in the one attempt using 17-day vernalization. A decrease in temperature during treatment apparently can be counteracted by an increase in moisture content of the seed, within limits.

Experiment 2. Marquis wheat was vernalized using the formula 8:3:60, developed in the preceding experiment, and grown in 8-inch pots in the laboratory at Saskatoon. The natural daylight was supplemented by electric lights of 200 watts, suspended 12 inches above the plants, to give a total of 15 hours of light per day. Soil temperature readings were taken twice a day, at 9 A.M. and 4 P.M. The average morning reading was 15° C. and the average afternoon soil

temperature was 20° C. The date of sowing was October 23. The number of vernalized plants was twenty-four and controls twelve.

The vernalized plants (*a*) emerged 2 days earlier; (*b*) formed each of the seven leaves at least 2 days earlier; (*c*) headed an average of 5.5 days earlier; (*d*) flowered an average of 7 days earlier; (*e*) ripened 4 days earlier; and (*f*) had a 1000-grain weight 10.37 per cent greater than the controls.

Experiment 3.—The formula 8:3:60 was used to vernalize Marquis wheat at Chicago during the summer of 1939. Three vernalized plants were grown in each of eighteen 6-inch pots and three control plants in each of twenty-one pots.

The wheat, the previous history of which was unknown, did not respond to treatment as did that of the previous year. In the term of the experiment, July 3 to September 3, 46.4 per cent of the treated plants had flowered in an average of 53 days. Of the controls 44.4 per cent flowered in an average of 53.5 days. Treatment increased the number of flowerings but accelerated maturity by only 0.5 day.

In a repetition of the experiment begun August 3, the vernalized grain flowered in an average of 49.7 days and the control in 52.3 days. The acceleration of flowering resulting from vernalization was 2.6 days. In both the 1938 and 1939 experiments the flowering time was shorter and the effects of vernalization more pronounced in later plantings.

The acceleration of flowering caused by vernalization of from 8 to 18 days in 1938 and but 0.5 to 2.6 days in 1939 when two different samples of vernalized Marquis wheat were grown in the greenhouse under similar conditions is perhaps due to differences in the histories of the wheats prior to treatment. The wheat used in 1939 may have been naturally vernalized to some extent during ripening and thus did not respond to the same degree to further vernalization. The result of the 1939 experiment suggested the problem of vernalization of spring wheat prior to reproductive maturity, which is being investigated at the present time.

PHOTOPERIODIC RESPONSE OF VERNALIZED SPRING CEREALS

Many workers in photoperiodism have determined that some cereals respond to increased photoperiods by an acceleration in

flowering. An experiment was conducted in 1934 to test the comparative response of vernalized and unvernallized spring cereals to continuous illumination.

Marquis wheat, Banner oats, O.A.C. barley, and Prolific rye were vernalized for periods of 5, 7, 13, 16, 17, 18, and 23 days using a temperature of 3° C. and 33 per cent added moisture. Five seeds of each set of cereals were planted in each of two 10-inch pots, filled with loam soil. The plants were grown in a greenhouse the temperature of which was uncontrolled. One complete set (two pots of each

TABLE 4
RESPONSE OF VERNALIZED AND UNVERNALLIZED SPRING CEREALS
TO INCREASED PHOTOPERIOD

GRAIN	VER- NALI- ZATION FORMU- LA	DAYS TO FLOWER						ACCELERATION DUE TO VERNALIZATION				DECREASE IN DAYS TO FLOWER DUE TO CONTINU- OUS ILLU- MINATION	
		GREENHOUSE				FIELD		GREENHOUSE		FIELD			
		VER- NAL- IZED 24 HOURS LIGHT	CON- TROL 24 HOURS LIGHT	VER- NAL- IZED NOR- MAL LIGHT	CON- TROL NOR- MAL LIGHT	VER- NAL- IZED	CON- TROL	CON- TINU- OUS LIGHT	NOR- MAL LIGHT				
		VER- NAL- IZED	CON- TROL	VER- NAL- IZED	CON- TROL	CON- TINU- OUS LIGHT	NOR- MAL LIGHT						
Marquis wheat .	23:3:33	36	37	46	50	59	61	I	4	2	10	13	
Banner oats . . .	13:3:33	35	36	46	49	67	68	I	3	I	11	13	
O.A.C. barley . .	13:3:33	35	35	54	58	65	66	o	4	I	19	23	
Prolific rye . . .	13:3:33	29	29	36	40			o	4		7	11	

cereal for each vernalizing formula) was exposed to normal illumination and another complete set to continuous illumination. Daylight was supplemented by electric lights of 500 watts suspended 4 feet above the tops of the pots.

The plantings, seven in all, were completed on May 5, 1934. Two controls were used, one of seeds germinated for 24 hours at 15° C. and the other of ungerminated, unchilled seeds. The experiment was conducted at Saskatoon.

In all cases the germinated and ungerminated controls flowered simultaneously. TAYLOR and COFFMAN (19) report a similar result in their experiments with spring oats.

The treatments which gave the greatest acceleration in time to flower (extrusion of anthers), compared with controls grown in the same photoperiod, are given together with flowering data in

table 4. Data from the field experiment of 1934 are included for comparison. The 13:3:33 treatment resulted in the shortest vegetative period for Banner oats, O.A.C. barley, and Prolific rye. Marquis wheat, however, flowered in the shortest time when vernalized according to 23:3:33.

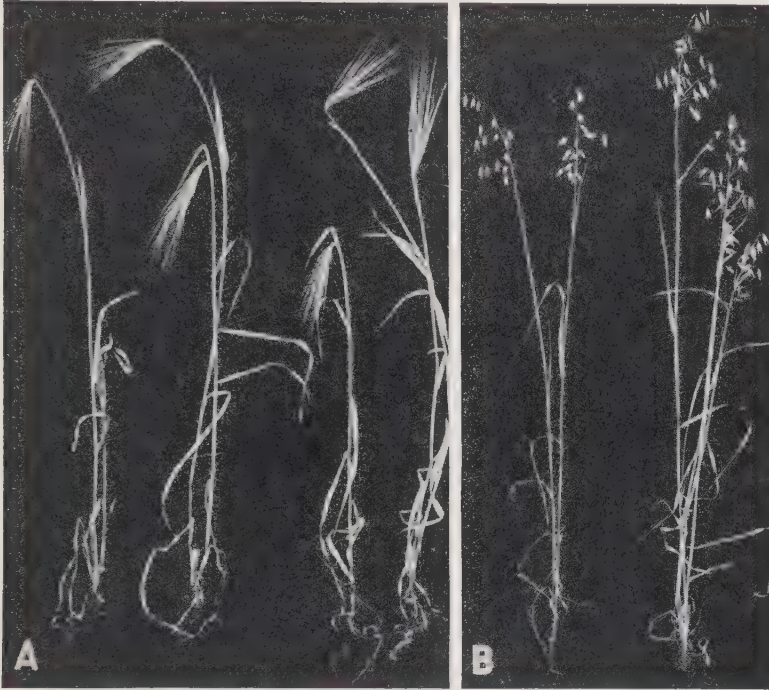


FIG. 1.—A: O.A.C. barley grown in normal illumination. Left to right: control 18 days; vernalized 18 days; control 7 days; vernalized 7 days. B: Banner oats grown in normal illumination. Left, control; right, vernalized 13 days.

In each case the acceleration of flowering caused by vernalization, compared with controls grown under the same light conditions, was less in continuous light than in normal illumination. Untreated plants reacted more than treated plants to increased illumination. The cereal whose flowering was accelerated most (19 days when vernalized, 23 days when untreated), due to a photoperiod of 24 hours, was O.A.C. barley; the least responsive was Prolific rye. Other responses are given in table 4.

Grains were produced very sparingly, in some cases having as few as six kernels a head. An estimation of possible yield, however, could be obtained from the size and number of heads. Vernalization resulted in greater height, greater stem diameter, larger average num-



FIG. 2.—*A*: prolific rye grown in normal illumination. Left, control; right, vernalized 16 days. *B*: Marquis wheat. Left to right: vernalized 18 days, grown in continuous illumination; control 18 days, grown in continuous illumination; vernalized 18 days, grown in normal illumination; control 18 days, grown in normal illumination.

ber of tillers, and larger heads than controls when grown under both normal and continuous illumination (figs. 1, 2). Plants receiving 24 hours of light were taller than those grown under normal illumination, the increased height being due to lengthened internodes.

A comparison of time for flowering in the field and in the greenhouse under the same photoperiod indicates, for all cereals grown, that vernalization accelerated maturity more when the plants were

grown at the higher temperature of soil and air in the greenhouse. This possible correlation between response to vernalization and soil temperature was investigated in the experiments described in the next section.

SOIL TEMPERATURE IN RELATION TO VERNALIZATION

To determine whether a correlation existed between soil temperature and vernalization response, vernalized Marquis wheat was grown at Chicago at various soil temperatures during 1937, 1938, and 1939. The method for each year is given in table 5.

TABLE 5
METHODS FOR GROWTH OF VERNALIZED WHEAT AT
VARIOUS SOIL TEMPERATURES

YEAR	VERNALIZATION FORMULA	PLANTED	HARVESTED	INITIAL GROWTH	SOIL TEMPERATURE (°C.)	REPLICATE POTS, EACH TEMPERATURE	TOTAL PLANTS, EACH TEMPERATURE	DURATION OF EXPERIMENT (DAYS)
1937.....	11:3:40	July 1	Aug. 27	Five days at 26° C., then at temperatures given	22°, 33°, 44°	3	15	57
1938.....	8:5:40	July 9	Aug. 26	Given temperatures from the start	22°-44° in 2° intervals	3	15	48
1939.....	8:3:60	July 3	Sept. 11	As 1937	22°, 26°, 30°, 34°, 38°, 42°	5	15	70

The vernalized and control wheats were planted in loam soil in 2-gallon porcelain containers. Five plants were allowed to grow in each pot during the 1937 and 1938 experiments and but three in 1939. The containers were immersed to within 2 inches of their tops in water. The temperature of the water was thermostatically controlled with the exception of the tank at 22° C. The lowest temperature that could be obtained by using continuously running water from the water mains was 22° C., and the temperature of the water (and hence of the soil in the containers) rose at times to 24° and fell to 21° C. In the other soil temperature tanks the fluctuations were $\pm 0.5^{\circ}$ C.

The temperature of the air in the greenhouse was uncontrolled and rose nearly every day to at least 100° F., falling at night to about

70° F. The plants were given sufficient water once a day to keep the soil in all pots moistened to approximately the same degree. More water had thus to be added to the pots at higher temperatures than at lower temperatures. Drains from the containers provided for removal of excess water.

Results of the three experiments were very similar, and only data for 1939 are tabulated in table 6. Significant differences are mentioned in the text.

TABLE 6
HARVEST DATA, SOIL TEMPERATURE EXPERIMENT, 1939

SOIL TEMPERATURE °C.	VERNALIZED OR CONTROL	No. PLANTS HARVESTED	AVERAGE DRY WEIGHT (GM.)			TOP/ROOT RATIO	GREATEST AVERAGE HEIGHT (IN.)	GREATEST AVERAGE ROOT LENGTH (IN.)	AVERAGE NO. OF TILLERS/PLANT	TOTAL HEADS IN 70 DAYS	AVERAGE HEADS PER PLANT	TIME FOR FIRST FLOWERING IN DAYS	AVERAGE TIME FOR FLOWERING IN DAYS	ACCELERATION OF FLOWERING IN DAYS
			Tops	Roots	Total									
22...	V	15	1.45	0.50	1.95	2.9	25.3	17.3	4.7	7	0.47	65	67.6	-1.3
		15	1.61	0.40	2.01	4.0	25.0	12.2	4.3	6	0.40	63	66.3	
26...	V	15	1.10	0.21	1.31	5.5	22.7	12.1	3.7	12	0.80	48	50.8	4.3
		13	0.90	0.23	1.19	4.2	22.5	12.1	3.5	10	0.77	60	64.1	
30...	V	12	0.38	0.07	0.45	5.4	19.6	10.1	2.5	3	0.25	52	58.3	2.3
		10	0.62	0.11	0.73	5.6	20.1	9.9	2.3	11	1.10	54	60.6	
34...	V	14	0.29	0.04	0.33	7.2	16.3	5.4	1.9	6	0.43	48	53.0	2.0
		14	0.21	0.03	0.24	7.0	16.4	4.4	1.6	4	0.30	51	55.0	
38...	V	15	0.15	0.03	0.18	5.0	12.4	3.3	1.4	4	0.27	52	55.5	6.5
		14	0.14	0.03	0.17	4.7	11.5	3.3	1.3	3	0.21	62	62.0	
42...	V	8	0.10	0.028	0.128	3.6	10.9	2.9	1.3					
		8	0.06	0.013	0.073	4.6	7.2	2.3	1.4					

As a rule the average height of the vernalized grain, as measured from the soil level to the tip of the longest leaf of the leading tiller, was slightly greater than controls at all soil temperatures throughout the growing period. Figure 3 expresses graphically the average growth of treated and untreated wheat from the age of 5 days to 35 days at the various soil temperatures. The vernalized plants were 3.7 inches taller than the controls at 42° C. and showed a greater dry weight. Less dying of leaves occurred in the treated plants. In the 1937 experiment all control plants grown at 44° C. died within 35 days, but four vernalized plants survived the 57 days, and two headed

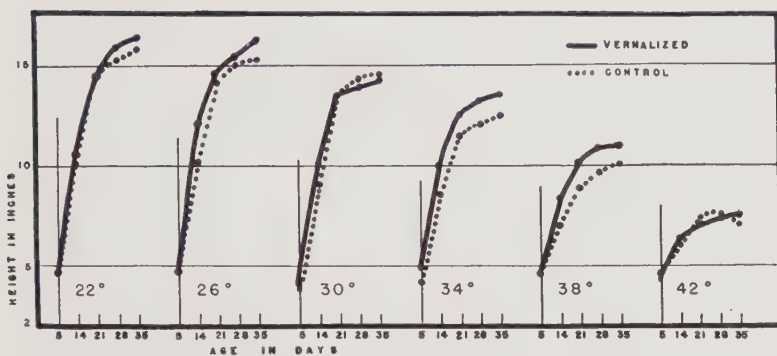


FIG. 3.—Average growth of vernalized and control Marquis wheat at soil temperatures of 22° to 42° C., from age of 5 to 35 days.

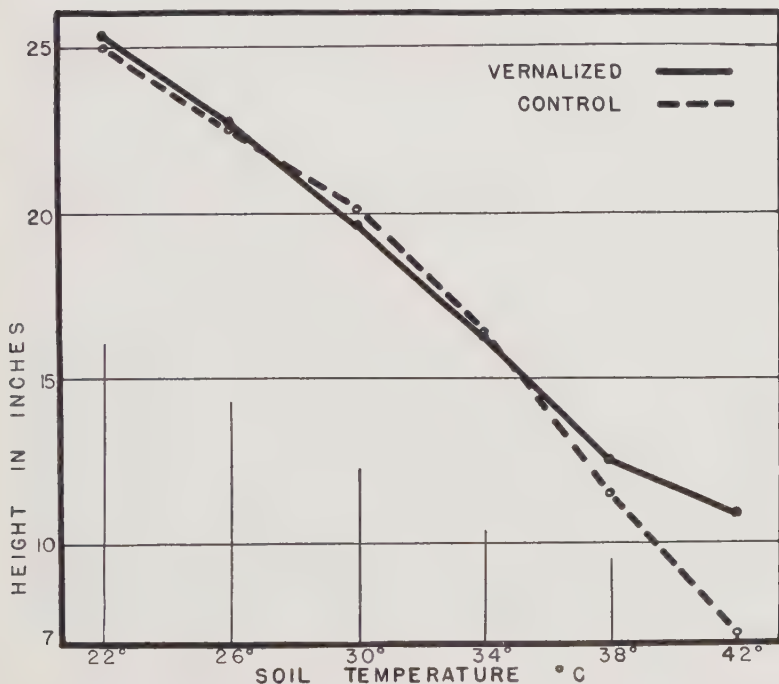


FIG. 4.—Heights of vernalized and control Marquis wheat grown at soil temperatures of 22° to 42° C., at age of 70 days.

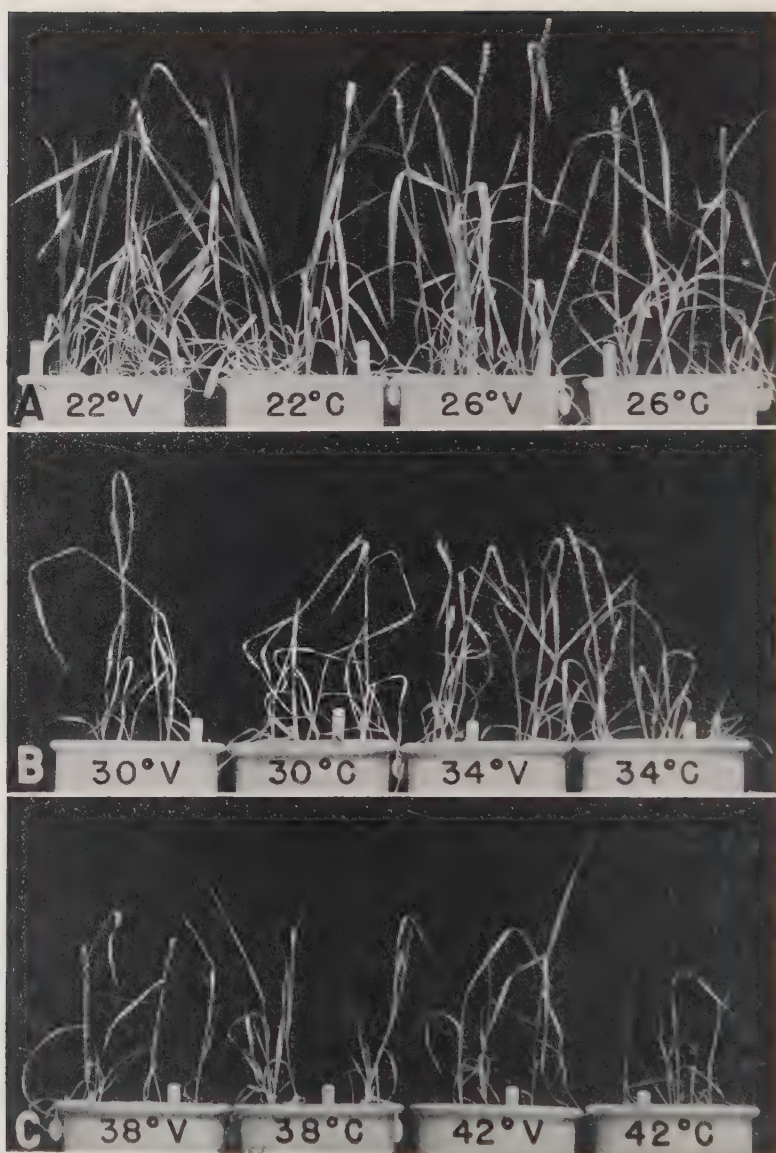


FIG. 5.—Representative plants of vernalized and control Marquis wheat grown at soil temperatures of 22° to 42° C., at age of 70 days. V, vernalized; C, control.

and produced grain. The height of the plants at 70 days, when they were harvested, is shown in figure 4, while figure 5 shows representative plants from each of the six soil temperatures.

The average greatest root length of treated plants was also greater than that of controls at all temperatures, although the comparative extent of the root systems varied somewhat. Roots decreased in length and extent with increasing soil temperatures.

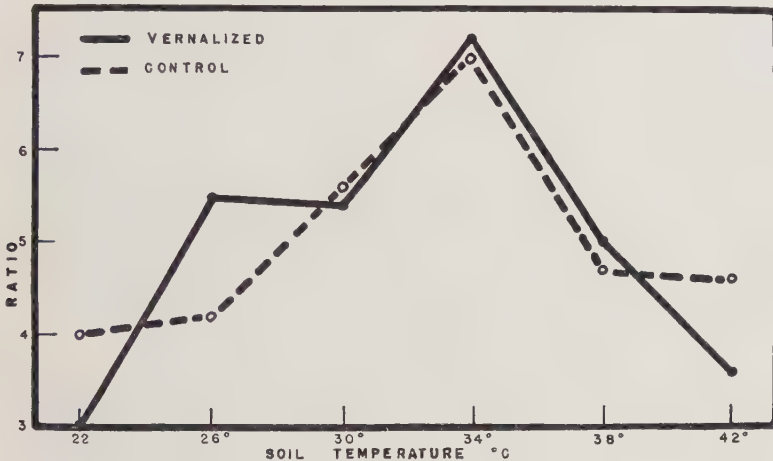


FIG. 6.—Top/root ratios of vernalized and control Marquis wheat grown at soil temperatures of 22° to 42° C., based upon dry weights at age of 70 days.

The number of tillers was greater on vernalized than on untreated plants, the average increase in the three experiments being 5 to 18 per cent.

No definite statement can be made from the results obtained as to the comparative dry weight behavior of the treated and untreated plants. Dry weights of both were less as temperatures increased. In both vernalized and unvernallized plants there was a rise in the top/root ratio as the temperature increased from 22° to 34° C., followed by a fall at higher temperatures (fig. 6). The high ratio indicates that the tops suffered comparatively less than the roots as the temperature rose to 34° C., but above this temperature the reverse is true.

The flowering behavior in 1937 and 1939 was similar. In 1937 the

control and vernalized plants flowered simultaneously in an average of 57 days when grown at a soil temperature of 22° C. At 33° C. the vernalized plants flowered in 48 days, the controls in 51 days. Two of the vernalized plants that survived at 44° C. flowered in 39 days. All control plants had died within 35 days. In 1939 both control and treated plants flowered in a shorter time as the soil temperature rose from 22° to 34° C. (table 6). Above 34° C. the flowering time was increased or earing prevented. The greatest acceleration of flowering resulting from treatment (6.5 days) occurred in plants grown at a soil temperature of 38° C.

In 1938 the vernalized plants flowered simultaneously at all soil temperatures from 22° to 40° C. The average time was 46 days. The controls had not headed within 48 days, and judging from appearance, would not have done so until at least 55 days. Vernalization accelerated maturity at all temperatures by approximately 10 days. A greater number of flowerings occurred, in the duration of the experiment, among vernalized plants grown at soil temperatures between 34° and 40° than at temperatures of 22° to 32° C.

The flowering behavior thus showed earlier flowering of both vernalized and control plants as the soil temperatures increased from 22° to 34° C.; and greater acceleration of flowering of vernalized plants over controls, or a greater number of flowerings in a given period, at higher soil temperatures.

The survival and flowering of some of the vernalized plants at a soil temperature of 44° C., their greater dry weight and smaller amount of dying of leaves at high temperatures than controls, indicate that given adequate moisture vernalized plants withstand high soil temperatures to a greater degree than untreated plants.

DIAGNOSIS OF THE VERNALIZED STATE

As advance in development of a plant is not always evidenced by morphological changes, it would be of benefit to be able to detect the qualitative changes which mark the completion of a phase by some microchemical method. A preliminary attempt, using modifications of methods employed by BASSARSKAJA (1, 2) and RICHTER (18), was made to detect completion of the thermo-stage (vernalization) in Marquis wheat seeds.

Marquis wheat was vernalized using 8:3:60 and the seeds were tested, employing the following techniques, after being chilled 4 days and again at completion of vernalization. The control was seed to which 60 per cent water had been added and allowed to germinate for 24 hours at 20° C.

a) Ferric chloride-potassium ferrocyanide method:

1. Sections of embryo cut at a thickness of 75 μ , using a freezing microtome.
2. Sections mounted using a gelatin fixative.
3. Slides treated with 5% ferric chloride for 3 minutes, rinsed thoroughly several times with distilled water, then treated for 3 minutes with 5% potassium ferrocyanide.

Treated in this way the vernalized and unvernallized seeds showed differences in reaction. As the seeds passed toward completion of the thermo-stage (vernalization), the color of the growing point changed from yellow, through green with areas of yellow, to a final dark blue. Tissues other than the growing point stained blue from the start.

b) Change of the iso-electric point:

1. Embryos sectioned and mounted as previously (crushing the embryo tissue on a slide smeared with fixative also proved faster and satisfactory).
2. Slides treated for 15 minutes with McIlwain's buffer solutions of pH 3.6 to 7.0 with intervals of 0.2.
3. Sections stained for 5 minutes in an aqueous solution formed by mixing equal volumes of a 1% solution of eosin and a 1% solution of methylene blue at the moment of staining.
4. After staining, slides rinsed in distilled water and returned to buffer solutions for 6 hours.
5. At conclusion of the 6 hours the pH of the buffers was determined, using a Coleman glass electrode apparatus.

At pH 5.32 sections from unvernallized seeds stained blue. At the end of 4 days of chilling the blue color was obtained at pH 5.13. When vernalization had been completed the embryos stained blue at pH 4.88. Thus, interpreting the results as does GAVRILOVA (5), the iso-electric point changed from pH 5.32 to pH 4.88 during vernalization.

Discussion

The development of a plant (advance to reproductive maturity) consists of a continuous series of phases occurring in strict sequence, according to the theory of vernalization or phasic development. The conditions required for the phases differ and the progress of each phase depends on an entire complex of habitat factors. At least four such phases have been reported (9, 21). In cereals the first or thermo-phase requires a relatively low temperature for its completion. Light is unimportant during this phase. The second or photo-phase requires a high temperature and a long day.

The aim of vernalization treatment is to induce the young embryo, which has just started growth, to complete the first phase of development during that period when its nutrition is least dependent upon external factors. The progress of the thermo-phase depends on a complex of seed moisture, temperature, and aeration. The experiments with Marquis wheat indicate that within certain limits a higher temperature may compensate for a moisture content below the optimum.

Species and even varieties are specific in their requirements of vernalizing factors (21). Hannchen barley responded to 12:3:33 whereas the other cereals did not, the moisture content apparently being too low. Of 125 different treatments Marquis wheat responded most markedly to 8:3:60. Decreases in time to flower of Hannchen barley and of Marquis wheat agree essentially with the results of a number of other workers (3, 4, 20). The failure of some investigators (6, 15, 16, 19) to obtain acceleration of maturity when cereals were vernalized may possibly be attributed to an ineffective combination of vernalizing factors during treatment.

The majority of investigators obtained increases in yield when spring cereals were vernalized. Reported increases of between 15 and 20 per cent (3, 4, 15) were substantiated by my field trials which gave an increase of 21.04 per cent on 5-acre plots.

The findings by Fokeev and Vyrov (3, 4) that vernalization resulted in greater increases in yield compared with control grain in areas of drought are not in accord with the results of these experiments.

The results obtained in the experiments involving photoperiodic

responses of Marquis wheat agree with those of MCKINNEY and SANDO (14), who found that Marquis wheat completes its life cycle quickly with a long photoperiod and a temperature of 70° F. or more throughout its life cycle. These workers also found that Marquis wheat formed seed very sparingly when grown in the greenhouse.

KOSTJUCENKO (11) reports that vernalized and unvernallized plants of spring wheat headed 5-9 days earlier in Hibiny (24-hour day) than in Pushkin (18-hour day). Continuous illumination in the experiment on photoperiod response resulted in earlier heading of vernalized plants by 7-19 days and of unvernallized plants by 11-23 days. The acceleration of flowering caused by vernalization was less in continuous light than in normal day length.

The response to vernalization is undoubtedly connected with the type of thermo-phase and the degree to which this phase has been completed prior to seed maturity (7, 8, 11, 12, 23), yet the response to treatment is definitely correlated with the after-sowing conditions and place of test. Thus the response to vernalization (shortening of the vegetative period) and length of the phase are not identical, for the after-sowing behavior of plants is governed by the environment, which has a different effect on the development of vernalized and unvernallized plants sown simultaneously. The Marquis wheat grown at various soil temperatures and the vernalized cereals grown simultaneously in the field and greenhouse in 1934 illustrate this point.

While the response obtained was not so simple as that reported by RAZUMOV and SMIRNOVA (17) for Bokhara barley grown in the continuous natural sunlight of the Arctic Circle in the greenhouse and in the field, the explanation is probably similar—the effect of temperature on the thermo- and photo-phase of the cereals. The thermo-phase of the unvernallized cereals was retarded at the higher temperatures, while the photo-phase proceeded more slowly at the lower temperatures. The photo-phase of the vernalized plants likewise proceeded more slowly at the lower temperatures than at the high; but because the thermo-phase had been completed during the vernalization treatment the higher temperatures resulted in a rapid completion of the photo-phase, giving an acceleration of 6.5 days in flowering compared with controls at a soil temperature of 38° C.

Not all developmental phases are perceptible morphologically, and not all morphological changes are a direct result of the transition of one phase to another. The morphological manifestations of those qualitative changes which constitute a development phase are subsequent in time to these changes, and the manifestations depend entirely upon the environmental conditions. From this standpoint the significance of the so-called phenological phases in recording the progress of development is practically annulled (21). The detection of biochemical changes within the plant, however, may serve to indicate the completion of a particular phase.

The formation of Prussian blue in the tissues of the embryo when treated with ferric chloride and potassium ferrocyanide is due to the reaction



The absence of Prussian blue in the cells of the growing point of the unvernallized seed may be due to two things: the ferric chloride does not penetrate into the cells or is reduced to ferrous chloride in the tissues (1, 2). The latter suggestion is confirmed by the formation of Prussian blue on the addition of potassium ferricyanide to the slides originally staining green. The conclusion is that vernalization produces a change in the oxidation-reduction potential of the cells. The shifting of the iso-electric point upon completion of vernalization indicates that the albumino-lipoids are likewise involved.

All that can be said with any degree of certainty is that some internal readjustments preceding morphological differentiation in the promeristem constitute the essentials of the thermo phase (21). Although supplying little information regarding the exact nature of these changes, the diagnostic experiments suggest it is the physical and chemical properties of the protoplasm which are concerned, and as a matter of course, form the basis for diagnosis.

Summary

1. The reaction of Marquis spring wheat to vernalization was studied through a period of 6 years. Other spring cereals investigated included O.A.C. and Hannchen barley, Banner and Victory

oats, and Prolific rye. Treatment was successful when snow or mechanical refrigeration was used to maintain chilling temperatures. Acceleration of flowering due to vernalization did not exceed 2.3 days when the cereals were grown in the field, with the exception of Hannchen barley which flowered 7 days and ripened 5 days in advance of controls. For plants grown in the greenhouse, the vernalization formula 13:3:33 (13 days chilling at 3° C., the water added being 33 per cent of the original air-dry weight of the seeds) resulted in an acceleration of flowering of 4 days in the case of O.A.C. barley and Prolific rye and of 3 days in the case of Banner oats. Of 125 different combinations of vernalization factors tested, 8:3:60 gave the greatest acceleration of flowering of Marquis wheat when grown in the greenhouse, the greatest average acceleration being 21 days. Increases in yield due to treatment amounted to 21.04 per cent on 5-acre plots. Increase in percentage yield due to treatment was less in time of drought than when growing conditions were favorable. Air drying vernalized seed reduced the percentage germination by 9.5.

2. Acceleration of flowering of Marquis wheat, O.A.C. barley, Banner oats, and Prolific rye due to vernalization was less when grown under continuous illumination than in normal day length, compared with controls receiving the same photoperiod. Increasing the photoperiod from normal day length to 24 hours shortened the vegetative period of untreated plants by 11–23 days and that of vernalized plants by 7–19 days.

3. Marquis wheat was grown at soil temperatures ranging from 22° to 44° C. Behavior showed earlier flowering of both vernalized and control plants as the soil temperature rose from 22° to 34° C., and a greater acceleration of flowering or a larger number of flowerings, due to vernalization, in plants grown at higher soil temperatures. While the dry weights of control and vernalized plants decreased as the soil temperature rose from 22° to 44° C., the top/root ratio rose to a maximum at 34°, falling to approximately the original value as the soil temperatures rose above 34° C. The growth of vernalized Marquis wheat in the greenhouse, with its higher temperatures, likewise resulted in a greater acceleration of flowering than when grown in the field.

4. Two microchemical methods were used to diagnose the vernalized state of Marquis wheat. Treatment of embryos with ferric chloride and potassium ferrocyanide showed a change in the ability of the growing point to reduce ferric chloride as vernalization progressed. A shifting of the iso-electric point of the embryo tissues from pH 5.32 to pH 4.88 upon completion of vernalization was detected by the use of McIlwain's buffers and staining with eosin and methylene blue.

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